

Amendment and Response

Serial No.:09/884,894

Confirmation No.: 1710

Filed: June 19, 2001

For: BIFIDOBACTERIA AND SIDEROPHORES PRODUCED THEREBY AND METHODS OF USE

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Remarks

The Office Action mailed October 21, 2002 has been received and reviewed. Claims 29 and 41 having been amended, and claims 43 and 44 having been added, the pending claims are claims 9-15 and 20-44. Reconsideration and withdrawal of the rejections are respectfully requested

The specification has been amended at the paragraph beginning at page 11, line 12, to correct a grammatical error. The original wording of the specification stated that the replication of a microbe is considered to be inhibited when its doubling time is decreased. This is an obvious error because inhibition of replication would result in an increase in doubling time, not a decrease. This grammatical error has been corrected by deleting "decreased" at page 10, line 17, and "reduced" at page 10, line 19, and inserting "increased" therefor.

The amendment to claim 29 is supported by the specification at, for instance, page 9, lines 12-16, and page 12, lines 19-21.

New claim 43 is supported by the specification at, for instance, page 5, lines 25-26.

New claim 44 is supported by, for instance, page 7, lines 2-3, page 15, lines 23-24, and page 17, lines 5-13.

It is Applicant's position that new claims 43 and 44 are directed to elected subject matter.

Comment regarding rejections of record in first Office Action and not repeated in the present Office Action

The present Action dated October 21, 2002, examines claim 29 on the merits. In the first Office Action, dated January 11, 2002, claim 29 was rejected under U.S.C. §112, second paragraph, and under 35 U.S.C. §102(b) as anticipated by Mutai et al. Applicant notes that these rejections are not repeated in the Action dated October 21, 2002. As these rejections are not repeated in the Action dated October 21, 2002, it is Applicant's understanding that these rejections of claim 29 have been withdrawn.

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NOT SURE
ABOUT THIS
- N/A
FOR NEW
EVIDENCE
rejection
1/14

The 35 U.S.C. §112, First Paragraph, Rejection

The Examiner rejected claim 29 under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for *Bifidobacterium* strains RecB1, strain RecB4, strain J1, strain J2, strain J4, strain P1, strain 6A or strain 10A, does not reasonably provide enablement for any and all strains of *Bifidobacterium*. Specifically, the Action asserts that an undue burden of experimentation would be necessary for one of skill to determine which *Bifidobacterium* strain is capable of secreting a siderophore. This rejection is respectfully traversed.

According to the M.P.E.P., "[t]here are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is 'undue.' These factors include, but are not limited to: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure" (M.P.E.P. §2164.01(a)).

The rejection of claim 29 under 35 U.S.C. §112, first paragraph, enablement, is based on the assertion that "[t]he unpredictability in the microbiological arts is very high" (Action, paragraph bridging pages 3-4). The Examiner is reminded that "[i]t is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others. The examiner's analysis must consider all the evidence related to each of these factors, and any conclusion of nonenablement must be based on the evidence as a whole" (M.P.E.P. §2164.01(a)). The Examiner has improperly ignored the other factors. The evidence concerning the other factors is discussed below.

The Examiner is respectfully requested to note that the specification discloses working examples teaching how to isolate and identify *Bifidobacterium* strains that secrete a siderophore. Working example 1 discloses how *Bifidobacterium* strains were isolated. The specification further discloses at working example 2 the method used for detecting siderophore

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production by bifidobacteria. It is evident upon review of the working examples that the quantity of experimentation needed to make or use the invention based on the content of the disclosure is minimal, and essentially all of the experimentation required is provided by the inventor. Further, as admitted by the Action, "methods of screening bacteria are conventional in the art" (Action, page 4), thus the experimentation required is well within the level of one of ordinary skill.

The Action states that the "unpredictability in the microbiological arts is very high and one of skill in the art would not be capable of predicting which Bifidobacterium strain produces a siderophore" and then concludes "[t]herefore, undue burden of experimentation would be necessary for one of skill to determine which Bifidobacterium strain [is] capable of secreting a siderophore" (Action, paragraph bridging pages 3-4). The statement regarding the high level of unpredictability in the microbiological arts is not understood, especially in view of the teachings of the present application. As detailed in working example 2, 8 of 29 (27.5%) of the strains isolated in working example 1 produced a siderophore. The Examiner is requested to clarify how experiments that resulted in positive results 8 out of 29 times could be considered an indication that "unpredictability in the microbiological arts is very high," or be considered undue experimentation.

Applicant also disagrees with the statement that "one of skill in the art would not be capable of predicting which Bifidobacterium strain produces a siderophore" (Action, page 4). The issue is not whether a skilled person can predict which Bifidobacterium strain produces a siderophore, but whether the skilled person can determine if a Bifidobacterium secretes a siderophore in view of the teachings of the specification. As stated by the Examiner, "it is not conventional to screen for Bifidobacterium strains that secrete siderophores" but it is applicant's position that now, in view of the specification, a skilled person can determine if a Bifidobacterium secretes a siderophore.

For at least the reasons stated above, applicant respectfully requests reconsideration and withdrawal of the rejection of claim 29 under 35 U.S.C. §112, first paragraph, for lack of enablement.

*yes, by using
working ex. 2*

The 35 U.S.C. §102 and §103(a) Rejections

The Examiner rejected claim 29 under 35 U.S.C. §102(b) as being anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Yildirim et al. (J. Food Prot., 61(1):47-51 (1998)). This rejection is respectfully traversed.

According to MPEP § 2131 a "claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." The present invention is directed to a "composition comprising a Bifidobacterium that secretes a siderophore" (claim 29). Siderophores secreted by Bifidobacterium are disclosed in the present specification as inhibiting the growth of *Micrococcus luteus*, *E. coli*, *Lactococcus lactis*, and *Clostridium perfringens* (specification at page 20, lines 8-23). Yildirim et al. teach a Bifidobacterium that produces a bacteriocin. The Action asserts that this bacteriocin, bifidocin B, is a siderophore (see Action at paragraph bridging pages 4-5). Applicant respectfully disagrees with this assertion. Yildirim et al. teach that bifidocin B does not inhibit many strains of bacteria, including *Micrococcus luteus*, *E. coli*, *Lactococcus lactis*, and *Clostridium perfringens* (see Yildirim et al. at Table 1). Thus, the compound secreted by the Bifidobacterium disclosed by Yildirim et al. does not have the same properties as a siderophore of the present invention. Since the bifidocin B taught by Yildirim et al. does not have the same properties as a siderophore of the present invention, the bifidocin B is not identical to the siderophore of the present invention.

Since the disclosure of Yildirim et al. does not set forth each and every element of claim 29, Yildirim et al. cannot anticipate claim 29. Withdrawal of this rejection under 35 U.S.C. §102(b) is respectfully requested.

Regarding the rejection of claim 29 under 35 U.S.C. §103(a) as obvious over Yildirim et al., the burden is on the Office to establish a *prima facie* case of nonobviousness of the claimed invention. The three criteria that must be met (MPEP § 2143) are: (i) there must be a suggestion or motivation to modify the reference; (ii) there must be a reasonable expectation of success; and (iii) the prior art reference must teach or suggest all the claim limitations. It is respectfully argued that the Office has fallen far short of meeting this burden.

Yildirim
et al.
J. Food Prot.
61(1):47-51
(1998)
Bifidocin B
is a bacteriocin
not a siderophore
Yildirim et al.
do not teach
that Bifidocin B
inhibits the growth
of Micrococcus
luteus, E. coli,
Lactococcus
lactis, and
Clostridium
perfringens.

To begin with, this rejection is based on the premise that Yildirim et al. teach a Bifidobacterium that secretes a siderophore. As discussed above, the bifidocin B secreted by the Bifidobacterium disclosed by Yildirim et al. does not have the properties of the siderophores of the present invention, thus, the premise is false.

"To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references" (MPEP §706.02(j)). The cited document does not expressly or impliedly suggest the claimed invention. The Action further states that "if there is a difference between the reference and the claimed subject matter regarding this feature then the whole bacterium in the composition would have been expected to secrete a siderophore as claimed herein" (Action, last 5 lines of page 5). This is conclusory, and does not constitute a convincing line of reasoning as to why the skilled person would have found the claimed invention to have been obvious in light of the teachings of the references, especially since the Bifidobacterium of Yildirim et al. is unable to inhibit the strains inhibited by the claimed siderophore.

The Action further states that it "is believed to be an inherent feature of the reference that the whole Bifidobacterium secretes a siderophore and this being taught by the reference that the same would be comprised by a composition as also taught by the cited reference" (Action, page 5). The legal criteria for establishing inherency, and the reasons why the allegedly inherent characteristic does not necessarily flow from the applied art were discussed in detail in the last response, and the Examiner is requested to review those comments (see Response mailed April 11, 2002, from page 10, second full paragraph through page 11, first full paragraph). For the reasons of record in the Response mailed April 11, 2002, it is applicant's position that the doctrine of inherency cannot be used to supplement the deficiencies of Yildirim et al.

The Action also states "[t]he reference clearly teaches such peptide compounds and the inhibition of growth of indicator strains which is by Applicant's own disclosure an

indicator that a siderophore is produced or formed by Bifidobacterium" (Action, page 5). This statement seems to suggest that merely observing the inhibition of an indicator strain by a Bifidobacterium proves that the Bifidobacterium produces a siderophore. This assertion is wrong. A siderophore is defined as a compound that inhibits the replication of other microbes by depriving them of iron (specification, page 8, lines 20-21), and the inhibition of indicator strains described at example 2 also included the control of adding iron to the solution containing the indicator strains (see page 20, lines 15-18, and lines 22-23). Further, claim 29 does not recite "peptide." The Examiner is requested to note that a siderophore "can be a polypeptide, or other organic materials" (specification, page 8, lines 23-24), thus, the assertion that a siderophore is a peptide is incorrect.

In conclusion, the Action does not provide any motivation for modifying the cited document, or any indication as to why there would be a reasonable expectation of success. Further, since the cited document does not teach or suggest a composition comprising a Bifidobacterium that secretes a siderophore, not all the claim limitations are present. The Examiner is respectfully requested to reconsider and withdraw the rejection of claim 29 as obvious over Yildirim et al.

The Examiner further rejected Claim 29 under 35 U.S.C. 102(b) as being anticipated by Nisbet et al. (U.S. Patent 5,340,577). This rejection is respectfully traversed.

The present invention is directed to a "composition comprising a Bifidobacterium that secretes a siderophore" (claim 29). Siderophores secreted by Bifidobacterium are disclosed in the present specification as inhibiting the growth of E. coli and Lactococcus lactis (specification at page 20, lines 8-23). At page 6, the Action notes that "the inhibition is enhanced by a composition comprising Enterococcus and Bifidobacterium, note col. 4, lines 15-17" (Action, page 6). It is applicant's position that the composition to which Nisbet refers in this passage also includes other bacteria:

"[i]t is envisioned that this invention may be practiced with a probiotic having as few as three different bacteria, i.e. a single Lactobacillus species, one of Lactococcus lactis or Citrobacter freundii, and only one Enterococcus, Bifidobacterium, Propionibacterium or Escherichia species. However, in

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accordance with a preferred embodiment, enhanced control of Salmonella is achieved with a probiotic which includes both Enterococcus and Bifidobacterium species."

(Nisbet et al., col 4, lines 9-17), thus Nisbet et al. do not teach a composition including just Enterococcus and Bifidobacterium. Nisbet et al. also disclose that "[t]he bacteria also should not produce bacteriocins effective against the other above-mentioned bacteria of the probiotic" (Nisbet et al., col 3, lines 55-57), and implicit in this statement is the desire of Nisbet et al. that the growth of one bacteria in the probiotic not be inhibited by another bacteria of the probiotic. If the Bifidobacterium taught by Nisbet et al. secreted a siderophore, it would inhibit the growth of other bacteria of the Nisbet et al. probiotic. Thus, the Bifidobacterium of Nisbet et al. do not secrete a siderophore. In fact, in detailing what the bifidobacteria of the probiotic produce, Nisbet teaches "the bifidobacteria . . . should be effective for producing volatile organic acids including acetic, propionic, and/or butyric acids" (col. 3, lines 35-38).

The Action also attempts to rely upon the doctrine of inherency to support the contention that Nisbet et al. teach a Bifidobacterium that secretes a siderophore. The legal criteria for establishing inherency, and the reasons why the allegedly inherent characteristic does not necessarily flow from the applied art were discussed in detail in the last response, and the Examiner is requested to review those comments (see Response mailed April 11, 2002, from page 10, second full paragraph through page 11, first full paragraph). For the reasons of record in the Response mailed April 11, 2002, it is applicant's position that the doctrine of inherency cannot be used to supplement the deficiencies of Nisbet et al.

Since Nisbet et al. do not teach a Bifidobacterium that secretes a siderophore, the disclosure of Nisbet et al. does not set forth each and every element of claim 29, and therefore cannot anticipate claim 29. Withdrawal of this rejection under 35 U.S.C. §102(b) is respectfully requested.

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The 35 U.S.C. §103 Rejection

The Examiner rejected claim 29 under 35 U.S.C. §103(a) as being unpatentable over Nisbet et al. in view of Yildirim et al. This rejection is respectfully traversed.

It is respectfully submitted that the Office has not met its burden of presenting a prima facie case of obviousness. The Action does not provide any reasoning as to why a skilled person would have been motivated to combine the cited documents, or any indication as to why there would be a reasonable expectation of success. Further, as discussed above, neither Nisbet et al. or Yildirim et al. teach or suggest a Bifidobacterium that secretes a siderophore. Thus, the combination does not teach or suggest all the claim limitations.

Reconsideration and withdrawal of the rejection of claim 29 under 35 U.S.C. §103(a) as being unpatentable over Nisbet et al. in view of Yildirim et al. is respectfully requested.

Comment on statement made at page 7 of the Action

Applicant notes the Action states at page 7 that "All claims fail to be patentably distinguishable over the state of the art discussed above and cited on the enclosed PTO-892 and/or PTO-1449." It is Applicant's understanding that only claim 29 has been examined on the merits in this Action (see Action at, for instance, page 3, line 13). Thus, the assertion at page 7 of the Action cannot apply to any non-examined claim.

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Summary

It is respectfully submitted that the pending claims 9-15 and 20-44 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
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CERTIFICATE UNDER 37 CFR §1.10:

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The undersigned hereby certifies that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

By:

Sandy Truehart

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APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE

Serial No.: 09/884,894
Docket No.: 110.0129 0101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

The paragraph beginning at page 11, line 12, has been amended as follows:

The ability to secrete a siderophore can be measured by various methods, including testing the ability of the siderophore to inhibit the replication of a microbe that does not produce the siderophore. Such a microbe is referred to herein as an "indicator strain." The replication of an indicator strain is considered to be inhibited when the doubling time of the indicator strain on a particular media containing a siderophore is [decreased] increased relative to the doubling time of the indicator on the same media not containing the siderophore. Preferably, the doubling time of the indicator strain is [reduced] increased by at least about 10%, more preferably by at least about 50%. Most preferably, the doubling time of an indicator strain in the presence of a siderophore is undetectable.

In the Claims

For convenience, all pending claims are shown below.

9. A method for inhibiting the replication of a microbe in the gastrointestinal tract of an animal, comprising administering to an animal a *Bifidobacterium* that secretes a siderophore, and measuring the presence of a microbe that was present in the gastrointestinal tract of the animal prior to administration, where a decrease in the presence of the microbe in the animal after administration of the *Bifidobacterium* indicates inhibition of the replication of the microbe.
10. The method of claim 9 further comprising growing the *Bifidobacterium* under iron limited conditions before administration.

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11. The method of claim 10 wherein growing the *Bifidobacterium* under iron limited conditions comprises growth in the presence of an iron chelator.
12. The method of claim 9 wherein the animal is a human.
13. The method of claim 9 wherein the microbe is a prokaryotic microbe.
14. The method of claim 13 wherein the prokaryotic microbe is selected from the group consisting of *E. coli*, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Clostridium difficile*, and *Clostridium perfringens*.
15. The method of claim 9 wherein the gastrointestinal tract is the large intestine.
20. A method for establishing a *Bifidobacterium* flora in the gastrointestinal tract of an animal comprising administering to an animal a *Bifidobacterium* that secretes a siderophore, and measuring the presence of the *Bifidobacterium* in the gastrointestinal tract of the animal after administration.
21. The method of claim 20 further comprising growing the *Bifidobacterium* under iron limited conditions before administration.
22. The method of claim 20 wherein the gastrointestinal tract is the large intestine.
23. The method of claim 20 wherein the animal is a human.

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24. The method of claim 23 wherein the human is an infant selected from the group consisting of an immature infant, a premature infant, and a mature infant.

25. The method of claim 23 wherein the administration occurs after the human has undergone antibiotic therapy.

26. The method of claim 23 wherein the administration occurs after the human has undergone chemotherapy.

27. A method for preventing the replication of microbes in a food, the method comprising adding to the food a *Bifidobacterium* that secretes siderophore.

28. A method for decreasing the risk of colon cancer comprising administering to an animal a *Bifidobacterium* that secretes a siderophore, and detecting the presence of aberrant crypt foci in the colon of the animal, where a lower number of aberrant crypt foci compared to an animal not administered the *Bifidobacterium* indicates a decrease in the risk of colon cancer.

29. (Amended) A composition comprising a *Bifidobacterium* that secretes a siderophore, wherein the composition comprises substantially no free iron, comprises an iron chelator, or a combination thereof.

30. A method for obtaining a secreted siderophore from a *Bifidobacterium*, the method comprising incubating a *Bifidobacterium* under iron limited conditions, and isolating the siderophore.

31. A method for preparing a siderophore, the method comprising incubating a *Bifidobacterium* under iron limited conditions, and sterilizing the culture.

32. The method of claim 31 wherein the culture is sterilized by removing essentially all water from the culture.
33. A sterile composition comprising a siderophore obtained from a *Bifidobacterium*.
34. An isolated siderophore obtained from a *Bifidobacterium*.
35. An isolated siderophore that binds Fe²⁺, the siderophore obtained from a *Bifidobacterium*.
36. A method for decreasing an amount of free iron in a composition, the method comprising adding to a composition a secreted siderophore obtained from a *Bifidobacterium*.
37. A method for decreasing an amount of free iron in a composition, the method comprising adding to a composition an isolated siderophore obtained from a *Bifidobacterium*.
38. A method for inhibiting the replication of a microbe in a composition, the method comprising adding to a composition a secreted siderophore obtained from a *Bifidobacterium*.
39. A method for inhibiting the replication of a microbe in a composition, the method comprising adding to a composition an isolated siderophore obtained from a *Bifidobacterium*.
40. A method for altering the expression of a siderophore in a *Bifidobacterium*, the method comprising incubating under iron limited conditions a *Bifidobacterium* that does not secrete a siderophore, and selecting for a *Bifidobacterium* that replicates in the iron limited condition.

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41. (Amended) A composition consisting essentially of a *Bifidobacterium* that secretes a siderophore.

42. A sterile composition consisting essentially of a siderophore obtained from a *Bifidobacterium*.

43. (New) A composition comprising a *Bifidobacterium* that can secrete a siderophore, wherein the composition is for use as a probiotic.

44. (New) A composition comprising a *Bifidobacterium* that can secrete a siderophore, and a food.